Modulation Effects of Baduanjin Exercise on Subjective Cognitive Decline (BDJ)

NCT04009382

02/6/2025

**Biostatistical analysis plan**

For all outcomes in this proposal, when applicable, we will 1) compare differences between different groups (BDJ vs control) in older adults with SCD using a mixed model regression analysis and 2) apply regression analyses to explore the association among changes of different measurements, including rsFC/GMV, the cognitive assessments, blood markers, and HRV. Other covariates such as age, gender, GDS score, and total practice times based on practice log will also be included in the model. Please see the following for more details.

***Data analysis on cognitive and behavioral measurements*** For cognitive assessments, blood markers, and HRV, we will perform an intent-to-treat analysis with the data available using a mixed model regression analysis and separately investigate whether the likelihood of missing the session is related to the treatment group. Note that a subject's data can be included in the mixed model regression analysis even if the data is missing some points.

The regression model will include the subject as a random effect. Fixed effects will include the treatment group and time points (baseline, 3, and 6 months after treatment) and their interaction. Other covariates such as age, gender, GDS, and total exercise time (hours) will also be included in the model to reduce subject heterogeneity. Similar analyses will be applied to compare baseline and follow-up phase differences between the BDJ and control groups. In addition, multiple regression analyses will be applied to explore the association between the cognitive assessments and other measurements, including blood markers and HRV. Covariates, such as age and gender, will also be included in the model.

***Resting state fMRI data analysis*** Similar to our previous studies, resting-state functional connectivity analysis will be conducted using the CONN toolbox. During the preprocessing, images will be realigned, segmented, and co-registered to each subject's high-resolution T1 scan, which will be normalized to the standard Montreal Neurological Institute (MNI) template. Images will also be smoothed using a 6 mm full-width at half-maximum (FWHM) Gaussian kernel and filtered with a frequency window of 0.008-0.09 Hz. In addition to these steps, we will employ segmentation of grey matter, white matter, and cerebrospinal fluid (CSF) for the removal of temporal confounding factors (white matter and CSF). Finally, data will be submitted for motion correction using the artifact detection toolbox. Time points in subjects' scans will be marked as outliers if the global signal exceeds three standard deviations from the mean or if scan-to-scan motion exceeds 0.5 mm deviation.

We will use two seed regions: 1) bilateral hippocampus templates extracted from the AAL [153] using WFU-Pick Atlas software and 2) *a priori* dorsolateral prefrontal cortex (DLPFC) seed (peak coordinate: ± 36, 27, 29, with 5 mm radius), which has been used in previous studies. Functional connectivity measures will be computed between each of these seeds and every other voxel in the brain. First-level correlation maps will be produced by extracting the residual BOLD time course from each seed and by computing Pearson's correlation coefficients between that time course and the time courses of all other voxels in the brain. Correlation coefficients will be transformed into Fisher z-scores to increase normality and allow for improved second-level General Linear Model analyses.

Group analysis: Group analysis will be applied using the random effects model with SPM12. A factorial design module in SPM12 will be applied with two groups and three time points (baseline, 3 months, and 6 months after treatment). Specifically, we will compare differences before and after three and six months of treatment between the BDJ and control groups. In

addition, multiple regression analyses will be applied between the rsFC changes between baseline and 6 months after treatment, and we will examine corresponding cognitive assessments, blood markers, and HRV outcome changes across all subjects and within each group. Analyses will also be applied between the first treatment rsFC changes at the first scan session and cognitive outcome changes after 6 months to explore if we can use the rsFC change in the first treatment to predict the long-term treatment effect. A threshold of $p < 0.001$ and $p < 0.05$ small volume corrected will be applied for the region of interest (ROI). A threshold of $p < 0.005$ uncorrected and $p< 0.05$ familywise error (FWE) corrected will be used for non-ROI. Based on previous studies [25, 36, 156-157] and our hypotheses, the ROIs will include: MPFC, ACC, putamen, insula, prefrontal cortex, parietal cortex, medial temporal lobe, amygdala, and the brainstem.

***Voxel-based morphometry (VBM) analysis*** VBM will be performed using SPM12 running under a MATLAB suite. Similar to our previous study, all images will first be checked for artifacts, structural abnormalities, and pathologies. Then, the images will be segmented into grey matter (GM), white matter, and cerebrospinal fluid (CSF) and normalized using the high dimensional DARTEL algorithm. Subsequently, a group-specific template will be created to reduce variability between subjects. The template will then be used to normalize the images into the standard Montreal Neurological Institute (MNI) space using the “DARTEL Normalize to MNI Space” program and the “preserve amount” option to retain the volumetric data of the original images. Finally, spatial smoothing will be performed with an isotropic Gaussian kernel of 8mm full-width at half maximum [172].

Group analysis will be calculated using a random effects model. We will perform second level analyses using a factorial design module in SPM12 to explore the differences between the different groups. There will be two factors included in the analysis. The first factor will have two levels (Baduanjin group and the control group) and the second factor will have three time points (baseline, 3 months, and 6 months). Age, gender, and education will also be included in the model as covariates. Similar to a previous study, an absolute threshold of 0.1 will be used for masking. Total intracranial volume will be obtained by summing up the overall volumes of grey matter, white matter, and CSF. A threshold of $p < 0.005$ and $p < 0.05$ small volume corrected will be applied for the region of interest (ROI). A threshold of $p < 0.001$ uncorrected and $p < 0.05$ FEW corrected will be used for non-ROI. *ROI will be the same as that of resting state functional connectivity analysis.*

*Additional brain morphometry analysis* Similar to previous studies, we will compare the volume of the left and right hippocampus, caudate nucleus, thalamus, and other related areas using longitudinal Freesurfer data analysis. Specifically, we will use the automated procedure for labeling different brain structures and measuring volume, cortical thickness, and surface. This procedure has been previously described in detail [175] and has been validated in adults with MCI and AD. The automatic segmentations will be visually inspected for accuracy. The left and right hippocampal volumes, insula, caudate, and thalamus will be assessed using mixed model regression analysis. The mixed regression analysis will include the subject as a random effect. Fixed effects will include the treatment group and time points (baseline, 3 months, and 6 months after treatment). Intracranial volume will be included as a covariate in the model. In addition to

brain structure, whole brain volume analyses of the cortical and sub-cortical gray matter, white matter, and total intracranial volume will also be similarly assessed.